Gliomatosis cerebri: no evidence for a separate brain tumor entity

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Abstract: Gliomatosis cerebri (GC) is presently considered a distinct astrocytic glioma entity according to the WHO classification for CNS tumors. It is characterized by widespread, typically bilateral infiltration of the brain involving three or more lobes. Genetic studies of GC have to date been restricted to the analysis of individual glioma-associated genes, which revealed mutations in the isocitrate dehydrogenase 1 (IDH1) and tumor protein p53 (TP53) genes in subsets of patients. Here, we report on a genome-wide analysis of DNA methylation and copy number aberrations in 25 GC patients. Results were compared with those obtained for 105 patients with various types of conventional, i.e., non-GC gliomas including diffuse astrocytic gliomas, oligodendrogliomas and glioblastomas. In addition, we assessed the prognostic role of methylation profiles and recurrent DNA copy number aberrations in GC patients. Our data reveal that the methylation profiles in 23 of the 25 GC tumors corresponded to either IDH mutant astrocytoma (n = 6), IDH mutant and 1p/19q codeleted oligodendroglioma (n = 5), or IDH wild-type glioblastoma including various molecular subgroups, i.e., H3F3A-G34 mutant (n = 1), receptor tyrosine kinase 1 (RTK1, n = 4), receptor tyrosine kinase 2 (classic) (RTK2, n = 2) or mesenchymal (n = 5) glioblastoma groups. Two tumors showed methylation profiles of normal brain tissue due to low tumor cell content. While histological grading (WHO grade IV vs. WHO grade II and III) was not prognostic, the molecular classification as classic/RTK2 or mesenchymal glioblastoma was associated with worse overall survival. Multivariate Cox regression analysis revealed MGMT promoter methylation as a positive prognostic factor. Taken together, DNA-based large-scale molecular profiling indicates that GC comprises a genetically and epigenetically heterogeneous group of diffuse gliomas that carry DNA methylation and copy number profiles closely matching the common molecularly defined glioma entities. These data support the removal of GC as a distinct glioma entity in the upcoming revision of the WHO classification.

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Gliomatosis cerebri: No evidence for a separate brain tumor entity

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Running title:
DNA methylation and copy number profiles in gliomatosis cerebri

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ABSTRACT

Gliomatosis cerebri (GC) is presently considered a distinct astrocytic glioma entity according to the WHO classification for CNS tumors. It is characterized by widespread, typically bilateral infiltration of the brain involving three or more lobes. Genetic studies of GC have to date been restricted to the analysis of individual glioma-associated genes, which revealed mutations in the isocitrate dehydrogenase 1 (*IDH1*) and tumor protein p53 (*TP53*) genes in subsets of patients. Here, we report on a genome-wide analysis of DNA methylation and copy number aberrations in 25 GC patients. Results were compared with those obtained for 105 patients with various types of conventional, i.e., non-GC gliomas including diffuse astrocytic gliomas, oligodendrogliomas and glioblastomas. In addition, we assessed the prognostic role of methylation profiles and recurrent DNA copy number aberrations in GC patients. Our data reveal that the methylation profiles in 23 of the 25 GC tumors corresponded to either *IDH* mutant astrocytoma (n=6), *IDH* mutant and 1p/19q codeleted oligodendroglioma (n=5), or *IDH* wildtype glioblastoma including various molecular subgroups, i.e., *H3F3A*-G34 mutant (n=1), receptor tyrosine kinase 1 (RTK1, n=4), receptor tyrosine kinase 2 (classic) (RTK2, n=2) or mesenchymal (n=5) glioblastoma groups. Two tumors showed methylation profiles of normal brain tissue due to low tumor cell content. While histological grading (WHO grade IV *vs.* WHO grade II and III) was not prognostic, the molecular classification as classic/RTK2 or mesenchymal glioblastoma was associated with worse overall survival. Multivariate Cox regression analysis revealed *MGMT* promoter methylation as a positive prognostic factor. Taken together, DNA-based large-scale molecular profiling indicates that GC comprises a genetically and epigenetically heterogeneous group of diffuse gliomas that carry DNA methylation and copy number profiles closely matching the common molecularly defined glioma entities. These data support the removal of GC as a distinct glioma entity in the upcoming revision of the WHO classification.

Key words: Gliomatosis cerebri, DNA methylation profiles, genomic aberrations, *IDH1* mutation, *MGMT* promoter methylation, molecular classification

List of abbreviations used:

A_IDH, astrocytoma *IDH* mutant; CIMP, CpG island methylator phenotype; CNS, central nervous system; EGFR, epidermal growth factor receptor; GBM_G34, glioblastoma WHO grade IV *H3F3A*-G34 mutant subgroup; GBM_RTK1, glioblastoma WHO grade IV receptor tyrosine kinase 1 subgroup; GBM_RTK2, glioblastoma WHO grade IV receptor tyrosine kinase 2 (classic) subgroup; GBM_mes, glioblastoma WHO grade IV mesenchymal subgroup; GC, gliomatosis cerebri; H3F3A, H3 histone, family 3A; IDH, isocitrate dehydrogenase; MGMT, O^6-methylguanine DNA methyltransferase; mOS, median overall survival; MRI, magnetic resonance imaging; O_IDH, oligodendroglioma *IDH* mutant and 1p/19q codeleted; TP53, tumor protein p53; WHO, World Health Organization

INTRODUCTION

Gliomatosis cerebri (GC) is a rare glial neoplasm characterized by extensive infiltration of the brain involving three or more cerebral lobes [6]. Bilateral tumor growth is frequent, and GC may also extend to infratentorial structures and even the spinal cord. Histologically, GC corresponds to diffusely infiltrating, mostly astrocytic gliomas of World Health Organization (WHO) grades II, III or IV [6]. GC with histological features of oligodendroglial differentiation have also been reported but are less frequent [21]. Although the current WHO classification of brain tumors [6] lists GC as a separate glial entity, this entity is not well defined beyond the criteria mentioned above. The highly variable course of disease, with median survival of about 30 months after diagnosis and broad variation from a few months to >40 months [7,8,21], also challenges the view that GC comprises a distinct glioma entity. Moreover, molecular studies on GC have reported genetic alterations that are also common in diffuse and anaplastic astrocytic gliomas or glioblastomas, such as isocitrate dehydrogenese 1 (*IDH1*) mutation, tumor protein 53 (*TP53*) mutation and epidermal growth factor receptor (*EGFR*) amplification [4,5,7,9,11,13,14,18,21]. No GC-specific genetic alteration or molecular signature has been reported to date. Overall, data on genetic and epigenetic aberrations in GC are scarce, and systematic analyses on larger cohorts of patients with well-documented clinical annotation are missing. The purpose of the present study was therefore to apply genome-wide DNA methylation and DNA copy number profiling to a multicenter cohort of clinically well-annotated GC cases, and to compare the respective aberration profiles with those typically present in the more common and molecularly well-defined types of diffuse gliomas, i.e., diffuse astrocytic and oligodendroglial gliomas as well as glioblastomas. We thereby aimed to answer the question, whether GC is indeed a distinct glial tumor entity that is characterized by specific genetic and/or epigenetic aberrations.

PATIENTS AND METHODS

Patients and neuroimaging

The clinical databases of the Division of Clinical Neurooncology, University of Bonn, the Department of Neurology, University of Zurich, and the repository of the NOA-05 trial [7] were retrospectively screened for patients fulfilling the following criteria: (1) adult (>18 years old) patient, (2) histologically proven diffuse glioma corresponding to astrocytoma, oligoastrocytoma or oligodendroglioma WHO grade II or III, or glioblastoma WHO grade IV confirmed by central pathology review (G.R.), and (3) tumor extent meeting the WHO criteria of GC, i.e., widespread infiltration of three or more cerebral lobes on T2-weighted MRI confirmed by central radiological review (E.H., U.H.). To determine the number of central nervous system (CNS) regions involved, the frontal, temporal, parietal, and occipital lobes of both sides were regarded as separate regions. Also, the basal ganglia including thalamus of both sides each, brain stem and cerebellum, and spinal cord were regarded as separate regions. (4) For all patients to be included, paraffin-embedded tumor tissue had to be available for extraction of sufficient amounts of DNA for methylation profiling. All local Ethics Committees approved clinical data collection and molecular analyses. All MRIs were evaluated for the extent of T2 hyperintensities (number of lobes involved), involvement of both hemispheres and focal contrast-enhancement. As previously described [7], all patients were evaluated for the presence of bilateral symmetric GC, i.e., the involvement of the same number of lobes of both sides.

Histological classification and extraction of tumor DNA

All tumor samples were histologically classified according to the criteria of the WHO classification of tumors of the central nervous system [6|. Histology revealed an estimated tumor cell content of at least 60% in the investigated tissue specimens except for two cases with estimated tumor cell contents of approximately 20%. Tumor DNA was extracted from

formalin-fixed paraffin-embedded tissue sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany).

Array-based DNA methylation and copy number profiling

To evaluate molecular subgroups of GC, we performed comparative cluster analysis of DNA methylation profiles generated for 25 GC tissue samples using the Illumina Infinium® HumanMethylation450 beadchip technology ('450k array'). DNA methylation profiles obtained for the 25 gliomatosis cerebri cases were evaluated together with DNA methylation profiles of 105 other gliomas, including 45 isocitrate dehydrogenase (*IDH*) and H3 histone, family 3A (*H3F3A*) wildtype glioblastomas, 15 *H3F3A*-G34 mutant glioblastomas, 15 *H3F3A*-K27 mutant glioblastomas, 15 *IDH* mutant astrocytic gliomas, and 15 *IDH* mutant 1p/19q codeleted oligodendroglial tumors. DNA methylation profiles obtained from ten normal cerebral hemisphere tissue samples were also included in the analyses. Individual samples were normalized by performing background correction and dye bias correction (shifting of negative control probe mean intensity to zero and scaling of normalization control probe mean intensity to 10,000, respectively). As reported before [10,19], the following probe filtering criteria were applied: removal of probes targeting the X and Y chromosomes (n = 11,551), removal of probes containing a single-nucleotide polymorphism (dbSNP132 Common) within five base pairs of and including the targeted CpG-site (n = 24,536), and probes not mapping uniquely to the human reference genome (hg19) allowing for one mismatch (n = 9,993). In total, 438,370 probes were kept for analysis.

For unsupervised hierarchical clustering of the 140 samples, we selected the 13,248 most variably methylated probes across the dataset (using a cut-off of standard deviation >0.25). Samples were clustered using 1-Pearson correlation coefficient as the distance measure and average linkage (x-axis). Methylation probes were reordered by hierarchical clustering using Euclidean distance and average linkage (y-axis). Unscaled methylation levels were depicted

in a heatmap ranging from unmethylated state (beta-value: 0.0, blue color) to hemimethylated (0.5, white) and fully methylated state (1.0, red).

In addition to DNA methylation profiling, we performed copy-number variation (CNV) analysis with data from the 450k arrays using the 'conumee' package for the R statistical environment (available at: http://www.bioconductor.org/packages/release/bioc/html/conumee.html). For the detection of amplifications and chromosomal gains and losses, automatic scoring was verified by manual assessment of the respective loci for each individual profile [10,19]. The *MGMT* promoter methylation status and the presence of the glioma CpG island methylator phenotype (CIMP) were determined based on 450k array data [1,24].

Survival and prognostic factors

Overall survival was calculated according to Kaplan and Meier from the day of diagnosis of GC, i.e., the day when both histological and imaging criteria were fulfilled for the first time, until death. Differences between subgroups were compared using the logrank test. Prognostic factors derived from molecular subgroups or dichotomized imaging parameters were analyzed by univariate Cox regression analysis. Multivariate analysis was performed with parameters showing differences between the subgroups with a significance of 0.1 or less. The correlation between dichotomized imaging and molecular parameters on 2 x 2 tables was determined using the chi square test.

RESULTS

Patient characteristics

Queries of the databanks of the Division of Clinical Neurooncology, University of Bonn (n=8), the Department of Neurology, University of Zurich (n=6) and the NOA-05 trial repository (n=11) revealed 25 patients with an unequivocal diagnosis of GC. This required the involvement of three or more brain lobes as determined by reference neuroradiology, in conjunction with biopsy specimens showing a diffusely infiltrating glioma with sufficient tumor material for genome-wide methylome analysis. The 11 patients of the NOA-05 trial were included in a previous publication reporting on clinical outcomes, MRI characteristics and selected genetic alterations such as *IDH1* mutation and *MGMT* promoter methylation [7].

Individual patient data are provided in Table 1. Table 2 summarizes the patients' clinical and imaging characteristics. Median age at diagnosis was 50.3 years (range 23.6-76.9 years). One third of the patients demonstrated a glioblastoma histology upon biopsy, while oligodendroglial differentiation was less common (20%) (Table 2). Half of the tumors were particularly extensive tumors and involved six or more lobes; most of the patients (88%) had bilateral involvement at the time of first diagnosis. Focal contrast enhancement at diagnosis of GC (i.e. 'Type 2' GC) was seen in 14 of 25 patients (56%). Figure 1 shows neuroradiological features in selected cases representing different histological and molecular glioma entities. Of the 25 patients, 2 received no chemo- or radiotherapy, 3 had radiotherapy alone as primary therapy, 14 had alkylating chemotherapy (13 procarbacin/CCNU, 1 temozolomide) and 6 patients received combined radiotherapy with concomitant and adjuvant temozolomide.

450k methylation profiling

Large-scale DNA methylation profiling analysis using 450k beadchip arrays revealed distinct methylation profiles in GC (Figure 2). Twenty-three of 25 samples could be assigned to one of the previously described epigenetic subgroups [19,23] of diffuse gliomas including *IDH* mutant astrocytoma (A_IDH: n=6; 24%), *IDH* mutant and 1p/19q codeleted oligodendroglioma (O_IDH: n=5; 20%), *IDH* wildtype glioblastoma (GBM) receptor tyrosine kinase (RTK) subgroup 1 (GBM_RTK1: n=4; 16%), *IDH* wildtype glioblastoma RTK2 (classic) subgroup (GBM_RTK2: n=2; 8%), *IDH* wildtype glioblastoma mesenchymal subgroup (GBM_mes: n=5; 20%), and glioblastoma *H3F3A*-G34 mutant in the 24-year-old, youngest patient of the cohort (GBM_G34; n=1; 4%). In 2 of 25 tumors, i.e. the two tumors with low tumor cell content, the normal brain tissue background was too high for adequate assignment to a specific glioma-associated methylation group, i.e., the methylation profiles in these cases corresponded most closely to a normal brain profile. DNA methylation profiling analysis did not reveal a previously unreported new subgroup of glioma enriched in this GC cohort.

In 23 of 25 tumor samples both a histological classification and a distinct glioma-associated methylation profile were available (Table 1). With conventional histology, 10/23 tumors were classified as glioblastoma WHO grade IV while 13/23 tumors were classified as diffuse or anaplastic gliomas corresponding to WHO grade II or III, 5 of them with a significant oligodendroglial component. With the use of DNA methylation profiling, the number of GC demonstrating an *IDH* wildtype glioblastoma-associated methylation profile increased to 12 tumors. Three GC tumors diagnosed histologically as WHO grade II or III gliomas showed methylation and copy number profiles typical for *IDH* wildtype glioblastoma WHO grade IV. One GC tumor histologically classified as glioblastoma demonstrated a DNA methylation profile corresponding to *IDH* mutant astrocytic gliomas. A concordance between histological diagnosis and DNA methylation-based classification was found in 19 of 23 cases.

Survival and prognostic value of histology and molecular subgroups

The median overall survival (mOS) of the whole patient cohort was 34.6 months (95% CI 7.1-62.1 months). The course of disease was highly variable, with 26.2% of patients having an overall survival time of less than 12 months and 49.2% of patients surviving for more than 3 years. Histological grading (WHO grade IV vs. WHO grades II/III) was not prognostic. Median OS was 34.6 months (95% CI not assessable) in the WHO grade II/III group and 36.2 months (95% CI 7.6-64.9 months) in the WHO grade IV group (p= 0.62, logrank test; Fig. 3a). The separation of cases according to *IDH* mutation / CpG island methylator phenotype (CIMP) status yielded borderline significance (p=0.08) for survival curves with median OS of 36.2 months for patients with *IDH* mutant / CIMP positive tumors (95% CI 32.9-39.6 months) as opposed to 14.1 months for patients with *IDH* wildtype/CIMP negative tumors (95% CI 11.1-17.1 months). When the GC patients belonging to the GBM_mes or GBM_RTK2 subgroups were compared to all other patients, differences between the groups regarding overall survival were highly significant: Median OS was 37.1 months (95%CI 35-39.2) in the group of patients with GC tumors molecularly corresponding to Astro_IDH, Oligo_IDH, GBM_RTK1 or GBM_G34 subgroups but only 12.2 months (5.9-18.4 months) in the group of patients with GBM_mes or GBM_RTK2 subgroup tumors (p=0.002; Fig. 3b). Also, univariate Cox analyses (Table 3) confirmed a significant negative effect when GBM_mes or GBM_RTK2 glioblastomas were compared with all other tumors, while no significant effect was found for histological grading (WHO grade IV vs. WHO grade II and III) or the separation of all molecularly defined glioblastomas from any other tumors (Table 3). A comparison of overall survival between the 6 patients with Astro_IDH and the 5 patients with Oligo_IDH group tumors revealed no significant difference (p=0.32). This finding is likely due to the small numbers of patients in each group and the relatively short follow-up for 4 of 5 patients in the Oligo_IDH group.

MGMT promoter methylation and selected DNA copy number aberrations

The results of the *MGMT* promoter methylation analysis and DNA copy number variations (CNV) analysis for selected genes/chromosomal regions are summarized in Table 1. *MGMT* promoter hypermethylation was found in 14 of 22 evaluable tumors. The following further genetic alterations were frequently found in this GC cohort (Table 1): 1p/19q codeletion (5/24 tumors), chromosome 7 gain (9/24 tumors), focal deletion at 9p21 (*CDKN2A/B*) (7/24 tumors), and chromosome 10 loss (8/24 tumors). Univariate Cox regression analysis revealed that *MGMT* promoter methylation was a positive prognostic factor (Table 4), while chromosome 7 gain and *EGFR* amplification (detected in 3/24 tumors) were negative prognostic factors. In a multivariate Cox analysis (Table 4) only *MGMT* promoter methylation remained a significant prognostic factor with superior overall survival for patients with a tumor harbouring a methylated *MGMT* promotor (Fig. 3c). Codeletion of 1p/19q was not prognostic, likely due to the fact that 4 of the 5 patients were censored at rather short follow-up times between 14-17 months.

Imaging features: prognostic value and association with molecular findings

Bihemispheric involvement, involvement of infratentorial structures, presence of a contrast-enhancing lesion on the first MRI fulfilling GC criteria or high lesion load with involvement of six or more regions of the brain were not associated with worse prognosis on univariate Cox regression analysis (Table 5).

The presence of a methylated *MGMT* promoter, *IDH* mutation / CIMP positivity, chromosome 7 gain or *EGFR* amplification did not correlate with the key imaging features, i.e., initial contrast enhancement, involvement of 6 regions or diffuse-symmetric involvement ($p>0.05$ for all analyses, chi square test). The histological detection of a WHO grade IV tumor was significantly associated with the presence of a contrast-enhancing lesion ($p= 0.02$, Chi

square test) and a high lesion load with involvement of 6 or more regions (p=0.02). However, the prognostically relevant detection of a molecularly defined GBM_MES or GBM_RTK2 tumor was not significantly associated with contrast-enhancing lesions (p=0.28) or high lesion load with involvement of 6 regions (p=0.30).

DISCUSSION

In the present cohort of 25 patients with GC, genome-wide DNA methylation and copy number profiling data provided evidence that GC tissue specimens with sufficient tumor cell content can unequivocally be assigned to known molecularly defined subgroups of non-GC diffuse gliomas. There was no evidence for a distinct GC-specific molecular subgroup. *MGMT* promoter methylation was the only significant single gene prognostic factor in this GC cohort that included a high percentage of GBM patients (40% GBMs defined histologically, 50% GBMs defined molecularly) and patients with alkylating chemotherapy (80%) as part of their first-line therapy.

Large-scale DNA methylation profiling using Infinium® HumanMethylation450 beadchip arrays has proven very useful for the elucidation of biologically distinct subgroups of medulloblastoma [10], ependymoma [15], glioblastoma [2,12,19], as well as diffuse and anaplastic gliomas [16,17,23]. In the present study of GC patients, 450k analyses revealed that DNA methylation profiles corresponded to those of several previously defined molecular subgroups of gliomas. Most importantly, this analysis did not demonstrate evidence for a distinct GC signature. This finding is in line with previous publications reporting on single gene alterations [4,5,7,11,13,14] or CGH patterns [22] typical for other glioma entities in smaller series of GC samples. Admittedly, the number of cases analyzed in the present cohort was also relatively small due to the general rarity of GC and the fact that for this retrospective analysis a sufficient amount of tumor tissue was necessary. It cannot be excluded that the requirement for a suitable amount of tissue may have introduced some bias to the selection of patients for molecular profiling. Nevertheless, the lack of a single specimen in our series (beyond the two samples hampered by too high normal cell count) that could not be assigned to one of the known glioma subgroups raises doubt that in much larger cohorts of GC patients a substantial number of tumors could be identified that would form a distinct and not yet described molecular GC subgroup.

Epigenome-wide methylation profiling appears to be highly valuable for classification and prognostic purposes. In this GC cohort, 10 of 25 tumors were classified as glioblastoma WHO grade IV by histological analysis while DNA methylation profiling revealed IDH wildtype glioblastoma-associated DNA methylation signatures in 12/23 evaluable tumors. Thus, similar to data recently reported for diffuse and anaplastic astrocytic gliomas [3,16,20,23], 450k beadchip-based DNA methylation profiling may lead to a refined diagnosis of GC tumors. This appears to be both biologically and clinically meaningful, since methylation profiling but not focal histology allowed for the identification of a subgroup of GC with poor prognosis. This group consists of patients with *IDH* wildtype glioblastomas corresponding to the GBM_RTK2 and GBM_MES subtypes (Table 3, Figs. 2 and 3B). These two subtypes constitute the two most common glioblastoma groups in adult patients [19]. In contrast, classification and grading of the GC cases according to histological criteria alone was not prognostically relevant. As a cautionary remark, it has to be kept in mind that these results have been obtained in a relatively small cohort of patients: Small changes in the composition of the subgroups were sufficient to alter the statistical significance of the analysis.

MRI analysis in our cohort did not reveal any imaging parameter that was associated with the prognosis of the disease. This finding has to be validated in larger cohorts. So far, previous reports on imaging features with prognostic value [18] in GC could not be reproduced. Our own previous finding that the presentation with a diffuse-symmetric involvement is prognostic [7] could not be further validated in the present cohort, probably since only 2 patients in the present cohort had a diffuse-symmetric presentation. Importantly, the presence of a focal contrast-enhancing tumor mass or a high lesion load with 6 or more brain regions involved was not prognostically relevant. These two imaging parameters were only associated with WHO grade 4 histology, which was prognostically irrelevant in our series and did not correlate with any prognostically relevant parameter such as GBM_MES/GBM_RTK2 molecular profile, or *MGMT* promoter methylation status. Overall, it remains puzzling that

MRI features are disease-defining for GC but do not show any detectable relationship with prognostically relevant subgroups or molecular features.

In summary, the dataset provided here suggests that GC is not a separate glioma entity: (1) the subgroups defined by DNA methylation profiling are the same as in non-GC glioma; a separate GC profile could not be detected. (2) the prognostic factor(s) are similar to those in non-GC glioma and GC-specific prognostic factors could not be detected. (3) Although MRI features are essential for making the diagnosis of GC they appear to be of limited prognostic relevance. Although the patient numbers analyzed here were small, and larger series reproducing these results would be desirable, it should be considered that GC more likely reflects a particularly widespread growth pattern that may be detected in different types of histologically and molecularly well-defined glioma entities, and does not represent a distinct entity of its own. The factors that drive the particularly infiltrative phenotype leading to the GC growth pattern have yet to be elucidated. However, the results presented here do not justify maintenance of GC as a separate disease entity and support its deletion in the upcoming revised WHO classification of central nervous system tumors.

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CONFLICTS OF INTEREST

UH has received advisory board honoraria from Roche, Mundipharma and Novocure, speakers honoraria from Roche, Medac and Mundipharma, travel reimbursement from Roche and Medac, grant support from Roche; MG has received advisory board honoraria from Roche and Mundipharma, speakers honoraria from Novartis, Roche and UCB, travel reimbursement from Roche and Medac; JPS has received advisory board honoraria from Roche and Mundipharma, speakers honoraria and travel reimbursement from Medac and Roche, grant support from Merck; MW has received advisory board honoraria from Celldex, Immunocellular Therapeutics, Magforce, Isarna MSD, Merck, Northwest Biotherapeutics, Novocure, Pfizer, Roche and Teva, Grant support from Acceleron, Actelion, Alpinia Institute, Bayer, Isarna, MSD, Merck, Novocure, PIQUR and Roche. All other authors do not have any conflict of interest.

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TABLES

Table 1: Individual patient characteristics: demographic, imaging, histological and molecular features and outcome
See separate file

Table 2. Clinical and imaging characteristics.

		N (%)
Demographic data		
Age (median, range)	50.3 years (23.6-76.9 years)	
Gender	Male Female	12 (48) 13 (52)
Histological analysis		
Histology	Glioblastoma Anaplastic Astrocytoma Anaplastic Oligoastrocytoma Oligoastrocytoma WHO II Oligodendroglioma WHO II Astrocytoma WHO II	10 (40) 6 (24) 2 (8) 2 (8) 1 (4) 4 (16)
WHO grading	WHO grade II WHO grade III WHO grade IV	8 (32) 9 (36) 8 (32)
Oligodendroglial component	Yes No	5 (20) 20 (80)
MRI characteristics		
Number of lobes involved Median (range)	<6 $\geq$ 6	12 (48) 13 (52)
Infratentorial involvement	Yes No	5 (20) 20 (80)
Bilateral involvement	Yes No	22 (88) 3 (12)
Diffuse symmetrical involvement[a]	Yes No	2 (8) 23 (92)
Contrast-enhancing tumor mass on first MRI showing GC	Yes No n.a.	14 (56) 10 (40) 1 (4)

[a]according to Glas et al. [7]
Abbreviations: GC, gliomatosis cerebri; n.a., not assessed

Table 3. Univariate Cox regression analysis regarding the prognostic value of the histological and molecular tumor classification for overall survival.

	Median survival in months		**Risk ratio (lower-upper 95% CI)**	**P**
Univariate analysis	**Condition 1**	**Condition 2**	**Condition 1 vs. condition 2**	
Histologically defined GBM	yes	no		
	36.2	34.6	1.33 (0.43-4.2)	0.62
Any molecularly defined GBM	yes	no		
	14.1	36.3	3.18 (0.81-12.5)	0.097
GBM_RTK2 or GBM_MES	yes	no		
	12.2	37.1	9.83 (1.78-54.2)	**0.009**

Abbreviations: GBM, glioblastoma WHO grade IV

Table 4. Univariate and multivariate Cox regression analysis regarding overall survival and single genetic factors.

	Median survival in months		**Risk ratio (lower-upper 95% CI)**	**P**
Univariate analysis	**Condition 1**	**Condition 2**	**Condition 1 vs. condition 2**	
MGMT promoter status	methylated	unmethylated		
	>37.1	8,9	0.09 (0.02-0.44)	**0.004**
IDH / CIMP status	mutant / positive	wildtype / negative	0.31 (0.08-1.23)	0.08
	36.3	14.1		
1p/19q status				
	Codeleted	Not codeleted		
	>16.5	34.8	0.44 (0.05-3.6)	0.44
Chromosome 7 gain	yes	no		
	8.98	>36.3	6.32 (1.78-22.5)	**0.004**
EGFR amplification	Yes	no		
	7.9	36.3	10.1 (2.0-51.1)	**0.005**
9p21 deletion	yes	no		
	37.1	34.6	1.15 (0.33-4.0)	0.82
Chromosome 10 deletion	yes	no		
	12.2	36.3	2.6 (0.77-8.73)	0.12
CDK4 amplification	yes	no		
	36.2	34.6	0.52 (0.1-2.6)	0.42
MDM2 or *MDM4* amplification	yes	no		
	14.1	34.6	1.06 (0.28-4.1)	0.93
Multivariate analysis				
MGMT promoter status	methylated	unmethylated	0.16 (0.03-0.95)	**0.04**
IDH / CIMP status	mutant / positive	wildtype / negative	1.27 (0.14-11.6)	0.83
Chromosome 7 gain	yes	no	4.2 (0.49-36.3)	0.19
EGFR amplification	yes	no	3.0 (0.56-16.3)	0,20

Table 5. Univariate Cox regression analysis regarding the prognostic value MRI features for overall survival.

MRI appearance	HR	95%CI	P
Infratentorial involvement, yes vs. no	0.4	0.05 – 3.1	0.38
Involvement of 6 or more lobes, yes vs. no	1.08	0.33 – 3.5	0.9
Bilateral involvement, yes vs. no	0.67	0.08 – 5.4	0.71
Contrast enhancing lesions[a], yes vs. no	1.39	0.37 – 5.3	0.63

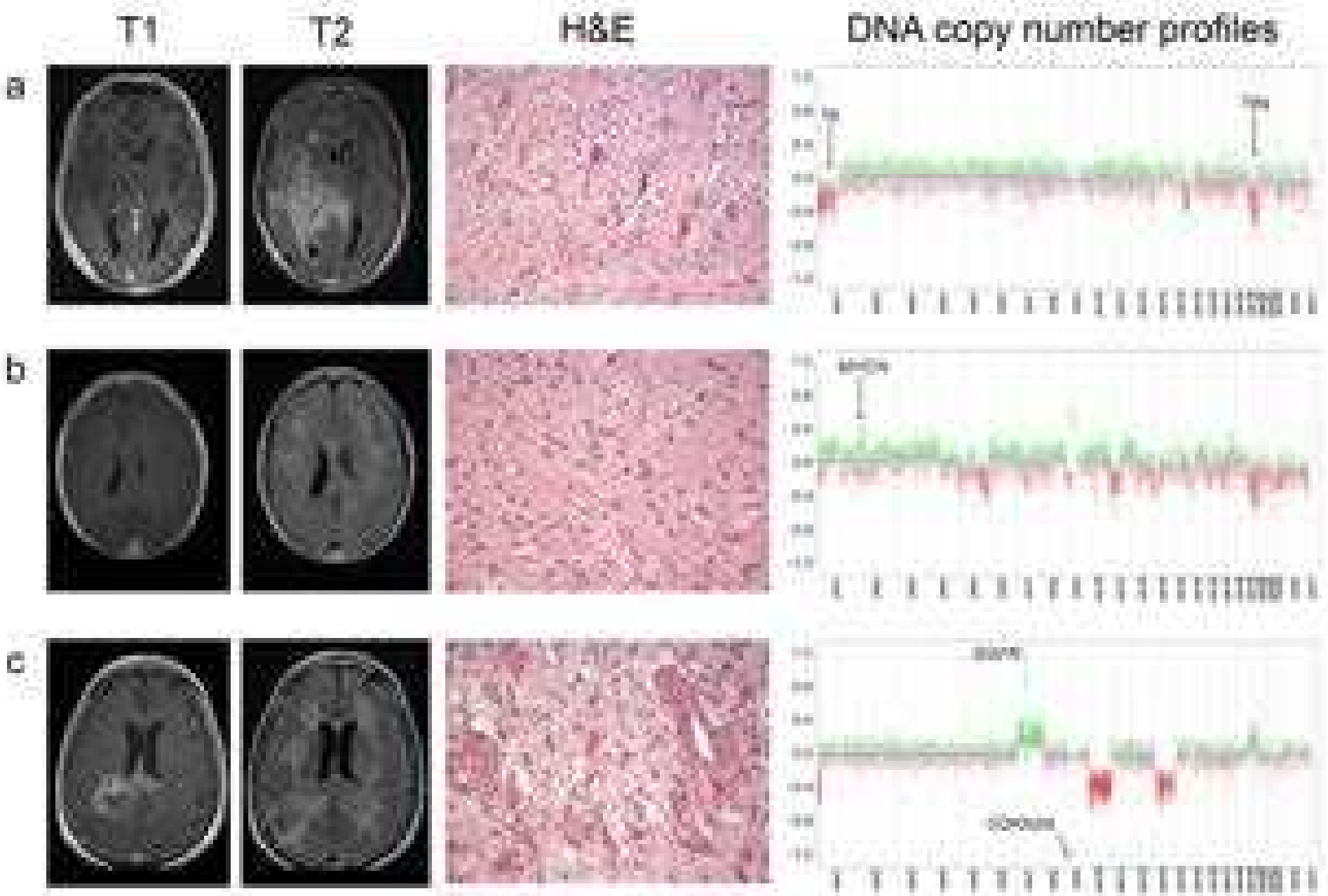
[a]on the first MRI fulfilling the criteria of GC
Abbreviations: HR, hazard ratio; CI, confidence interval

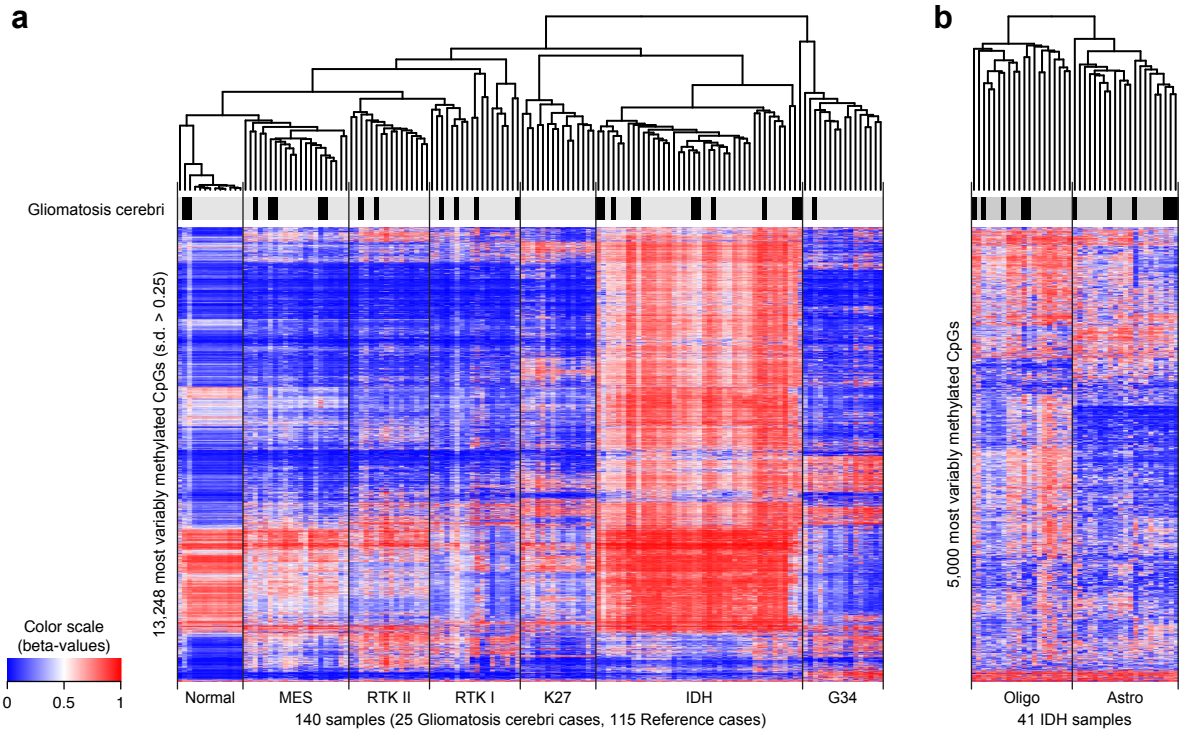
FIGURE LEGENDS

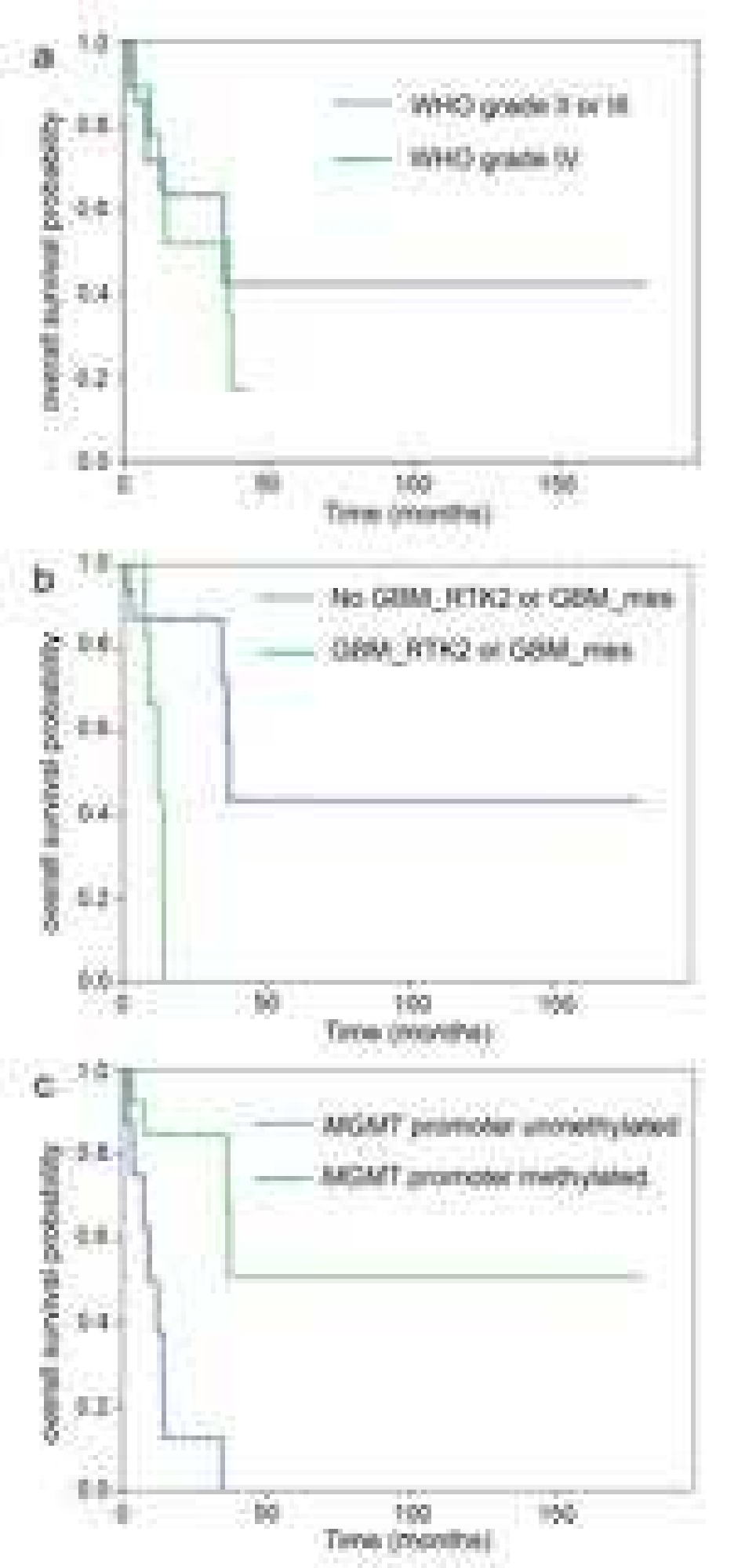
Figure 1. Examples of neuroradiological, histological and genetic findings in selected cases of GC. Shown are contrast-enhanced T1- and T2-weighted MRI images (left panel), corresponding histological features on biopsy specimens (middle panel) and DNA copy number profiles determined by 450K methylation array profiling (right panel). The represented cases correspond to patients having GC with *IDH* mutant and 1p/19q codeleted oligodendroglioma **(a)**, GC with *IDH* mutant diffuse astrocytoma **(b)**, or GC with *IDH* wildtype glioblastoma also demonstrating *EGFR* amplification, homozygous CDKN2A deletion and chromosome 10 deletion **(c)**. The histological pictures show hematoxylin-eosin (H&E) stained sections (original microscopic magnification: 400x). The copy number profiles represent gene dosages along chromosomes 1 to 22, X and Y. A stronger intensity of green or red colouring of probes indicates an increasing shift away from the baseline towards copy number gain or loss, respectively. Note 1p/19q codeletion in (a), *MYCN* gain in (b), and gains of chromosomes 7, losses of chromosome 10, homozygous deletion of *CDKN2A* as well as amplification of *EGFR* in (c).

Figure 2. Heatmap representation of an unsupervised hierarchical clustering analysis of 450k methylation array profiles obtained from 25 GC patients, 10 normal brain samples, as well as 105 previously reported non-GC gliomas of different molecular types used for comparison [19,23]. The non-GC cases corresponded to various molecular subtypes of *IDH* wildtype glioblastomas (GBM_RTK1, GBM_RTK2, GBM_mes, GBM *H3F3A*-G34 mutant, GBM *H3F3A*-K27 mutant) as well as *IDH* mutant astrocytic and oligodendroglial tumors. **(a)** Results obtained with the entire tumor cohort using the 13,248 most variably methylated probes (standard deviation >0.25). Note that the 25 GC tumors (red bars) do not form a distinct cluster but are distributed across the different non-GC glioma subtypes. **(b)** Results obtained in the subset of *IDH* mutant gliomas using the top 5,000 most variably methylated probes. Note that subsets of *IDH* mutant GC tumors (red bars) either cluster together with the *IDH* mutant astrocytic gliomas (n=6) or the *IDH* mutant and 1p/19q codeleted oligodendroglial tumors (n=5).

Figure 3. Overall survival probability according to histological **(a)** or molecular **(b-c)** features of gliomatosis cerebri (GC). **(a)** No significant difference in overal survival between GC patients with histologically diagnosed WHO grade IV glioblastoma vs. patients with WHO grade II or III gliomas (p=0.62 logrank test). **(b)** GC patients with RTK2/classic glioblastoma (GBM_RTK2) or mesenchymal glioblastoma (GBM_mes) demonstrate significantly shorter survival as vompared to patients with GC of other molecular subgroups (p=0.002, logrank test). **(c)** Prognostic stratification of GC patients according to *MGMT* promoter methylation status (p<0.001, logrank test).


T1
T2
H&E
DNA copy number profiles
a
b
c


a
b
Gliomatosis cerebri
13,248 most variably methylated CpGs (s.d. > 0.25)
Color scale
(beta-values)
0
0.5
1
Normal
MES
RTK II
RTK I
K27
IDH
G34
140 samples (25 Gliomatosis cerebri cases, 115 Reference cases)
5,000 most variably methylated CpGs
Oligo
Astro
41 IDH samples


a
WHO grade II or III
WHO grade IV
Time (months)
b
No GBM_RTK2 or GBM_mes
GBM_RTK2 or GBM_mes
Time (months)
c
MGMT promoter unmethylated
MGMT promoter methylated
Time (months)